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Recent pattern of antibiotic resistance in *Staphylococcus aureus* clinical isolates in Eastern India and the emergence of reduced susceptibility to vancomycin

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Abstract:

PURPOSE: We aimed to determine the recent pattern of antibiotic resistance and assess the vancomycin susceptibility profile of clinical *Staphylococcus aureus* in view of emerging reports of vancomycin creep, reduced vancomycin susceptibility (RVS), including heterogeneous vancomycin-intermediate *S. aureus* (hVISA) and vancomycin-intermediate *S. aureus,* and vancomycin resistance in *S. aureus* isolates.

MATERIALS AND METHODS: Consecutive, nonduplicate isolates of *S. aureus* between July 2015 and June 2016 were subjected to antimicrobial susceptibility testing using standard disk diffusion test or Etest as per the Clinical and Laboratory Standards Institute 2015. Detection of hVISA was done by glycopeptide resistance detection Etest according to the manufacturer's instructions in strains with vancomycin minimum inhibitory concentration of 1–2 μg/ml.

RESULTS: A total of 284 *S. aureus* were obtained from pus (175, 61.6%), respiratory tract (31, 10.9%), urine (27, 9.5%), blood (25, 8.8%), body fluids (18, 6.3%), and catheter tips (8, 2.8%). 127 (44.7%) isolates were methicillin resistant, and 158 (55.6%) were multidrug resistant. High resistance was observed to penicillin (81.7%), erythromycin (62.3%), and ciprofloxacin (52.1%), whereas the resistance was low to gentamicin (5.3%), rifampicin (8.1%), and doxycycline (9.5%). Two hundred and fifty-one (88.3%) isolates were fully susceptible to vancomycin, whereas 33 (11.6%) demonstrated RVS. All were uniformly susceptible to linezolid, tigecycline, and daptomycin.

CONCLUSIONS: A moderately high percentage of *S. aureus* isolates demonstrated RVS, which may limit its usefulness in methicillin-resistant isolates and may be associated with increased complications in methicillin-susceptible infections. In view of increasing glycopeptide resistance, the susceptibility status of vancomycin along with other antibiotics among clinical S. aureus isolates should be investigated periodically.

Keywords:

Heterogenous vancomycin-intermediate Staphylococcus aureus, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, vancomycin, vancomycin-intermediate *Staphylococcus aureus*

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Introduction

Staphylococcus aureus is an important pathogen in both community and hospital settings with manifestations ranging from relatively mild localized skin and skin-structure infections to life-threatening systemic infections, such as sepsis, necrotizing pneumonia, septic

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arthritis, endocarditis, and osteomyelitis.[1,2] Especially, the methicillin-resistant *S. aureus* (MRSA) constitutes one of the most serious contemporary challenges to treatment, being simultaneously resistant to several classes of drugs and being able to have a rapid global spread.[1,2] For MRSA and multidrug-resistant (MDR) *S. aureus* infections, vancomycin is considered the mainstay of antimicrobial therapy and has been the treatment of choice for serious MRSA infections since 1958.[3]

However, emerging reports of vancomycin minimum inhibitory concentrations (MIC) creep, reduced vancomycin susceptibility (RVS), and even vancomycin resistance in clinical MRSA isolates from different geographical regions are disturbing, because of minimal alternative therapeutic options.[3-6] This is because the clinical effectiveness of vancomycin may be compromised even against vancomycin-sensitive *S. aureus* (VSSA) strains whose MICs are within the Clinical and Laboratory Standards Institute (CLSI) susceptible range (≤2 μg/ml). Such heterogeneous vancomycin-intermediate *S. aureus* (hVISA) strains may evolve into VISA, leading to clinical treatment failure.[3,4] Recently, it has also been observed that high vancomycin MIC in methicillin-susceptible *S. aureus* (MSSA) is associated with poorer outcomes and increased complications in patients with *S. aureus* bacteremia and endocarditis.[7,8] Thus, determination of vancomycin MIC and the ability to accurately identify *S. aureus* isolates with reduced susceptibility to vancomycin is of paramount importance for several reasons such as successful clinical outcomes, the targeted treatment of MRSA bacteremia, or as a prognostic marker in MSSA bacteremia.

Because RVS in *S. aureus* is a complex phenomenon and difficult to detect in clinical microbiology laboratories, the CLSI in January 2006 redefined and updated the resistance breakpoints for vancomycin against *S. aureus* (susceptible, intermediate, or resistant when the vancomycin MIC is ≤2 μg/ml, 4–8 μg/ml, or ≥16 μg/ml, respectively) to increase the sensitivity in detecting the nonsusceptible isolates.[9] In addition, in 2009, the CLSI altered the guidelines for staphylococci such that disk diffusion was no longer an acceptable means for testing vancomycin susceptibility in these organisms.[10] For the detection of hVISA, though the population analysis profile–area under the curve (PAP-AUC) ratio is regarded as the gold standard test, it is relatively cumbersome, time-consuming, labor-intensive, and difficult to perform in a clinical diagnostic laboratory. Hence, newer tests such as the glycopeptide resistance detection (GRD) Etest and the macromethod Etest are being used recently.[11,12] With the emergence of RVS, various alternative therapeutic options have been advocated for the treatment of resistant *S. aureus* infections such as ceftaroline, tigecycline, linezolid, telavancin, and daptomycin, which have shown considerable clinical efficacy against *S. aureus* in various studies.[13,14]

Only a few studies from India have previously reported reduced susceptibility to vancomycin after the introduction of the revised CLSI guidelines.[15-19] Hence, we aimed to determine the recent pattern of antibiotic resistance, assess the vancomycin susceptibility profile, and ascertain other therapeutic options for clinical *S. aureus* isolates in Eastern India, which would help in choosing empirical antimicrobial therapy as well as in administering targeted therapy to the patients.

Materials and Methods

The study, approved by the institutional ethical committee, was conducted over a period of 1 year from July 2015 to June 2016 in a tertiary care research, referral, and teaching hospital in Eastern India. The hospital receives patients not only from the local areas but also from adjoining districts and states who are referred.

Bacterial isolates

Consecutive nonrepeat, nonduplicate *S. aureus* isolates obtained from patients with clinically diagnosed infections (one isolate per patient) whose clinical specimens were submitted to the department of microbiology by the treating physician were included in the study. The isolates were identified by colony characteristics, Gram stain reaction, and standard biochemical tests.[20]

Antimicrobial susceptibility testing, minimum inhibitory concentrations determination, and detection of heterogeneous vancomycin-intermediate *Staphylococcus aureus*

Susceptibility testing of *S. aureus* to the primary antimicrobial agents was performed by disk diffusion testing on Mueller-Hinton agar (MHA) as per the latest CLSI guidelines.[21] All the disks were procured from HiMedia, Mumbai, Maharashtra, India. MRSA were identified by cefoxitin (30 μg) disk testing and cefoxitin MIC by Etest (HiMedia, Mumbai, Maharashtra, India) as per the CLSI 2015 (zone diameter, sensitive ≥22 mm and resistant ≤21 mm; MIC, sensitive ≤4 μg/ml and resistant ≥8 μg/ml).[21] Inducible clindamycin resistance in isolates displaying erythromycin resistance was detected by the D-test.[21]

Susceptibility testing to vancomycin was performed to determine the MIC by both Etest (HiMedia, Mumbai, Maharashtra, India) and agar dilution with incorporation of intermediate dilutions in the agar dilution test for better correlation between the two methods of testing. In case of discrepancies between Etest and

agar dilution, the values of Etest MICs were deemed to be final because Etest has been found to be a more sensitive method for MIC determination and considered the best technique to assess exact MIC as it allows for visualization of small colonies around the zones of inhibition.[22] With Etest as the gold standard for susceptibility testing, detection of hVISA was done by GRD Etest on MHA with a 5% blood agar plate according to the manufacturer's instructions (HiMedia, Mumbai, Maharashtra, India) in strains with vancomycin MIC 1–2 μg/ml.[11,22] The test isolate was considered positive for hVISA if the Etest GRD strip result was ≥8 μg/ml for vancomycin or teicoplanin.[11,22] Isolates displaying either VISA (vancomycin MIC >2–<16 μg/ml) or hVISA (detected by GRD Etest) phenotype were designated as strains with RVS.[4]

Minimum inhibitory concentration was also determined for alternative therapeutic options such as linezolid, daptomycin, and tigecycline by Etest (HiMedia, Mumbai, Maharashtra, India) and interpreted as per the CLSI guidelines,[21] except tigecycline (for which CLSI currently does not provide interpretative zone diameters). *S. aureus* strains ATCC 25923, ATCC 51299, and ATCC 43300 were used as quality control strains for disk diffusion, MIC testing, and MRSA testing, respectively. Tigecycline susceptibility was interpreted as ≥19 mm zone diameter and ≤0.5 μg/ml MIC breakpoint as per the US Food and Drug Administration guidelines, respectively.[23] Strains with intermediate resistance were included in the percentage of resistant isolates. Multidrug resistance was defined as nonsusceptibility to at least one agent in three or more antimicrobial categories.[24]

Results

A total of 284 *S. aureus* were isolated during the study period with sample-wise distribution as follows: pus (175, 61.6%), respiratory samples (31, 10.9%), urine (27, 9.5%), blood (25, 8.8%), body fluids (18, 6.3%), and catheter tips (8, 2.8%). "Seventy-nine (27.8%) isolates were from the outpatient department (OPD), whereas 205 (72.2%) were from admitted patients. Of the 284 isolates, 87 were from dermatology, 76 from surgical, 74 from adult medical, 18 from pediatric and 29 from intensive care units. One hundred and sixty-six (58.4%) isolates were from male patients, whereas 118 (41.5%) were from females. The lowest and highest age at which *S. aureus* was isolated was from the pus sample of a 6-day-old male child and from the sputum sample of an 85-year-old male elderly patient, respectively. The underlying disorders of the patients are summarized in Table 1, with majority (65.8%) of patients having skin and skin-structure infections.

Table 1: Underlying disorders of the patients from whom *Staphylococcus aureus* were isolated (*n*=284)

Underlying disorder	Number of patients (%)
Skin and skin-structure infections[a]	187 (65.8)
Respiratory tract infection[b]	41 (14.4)
Genitourinary tract disease[c]	18 (6.3)
Neurologic diseases[d]	12 (4.2)
Malignancies[e]	12 (4.2)
Gastrointestinal pathology[f]	10 (3.5)
Undetermined	4 (1.4)

[a]Includes chronic nonhealing wound ulcer (64 patients), subcutaneous abscess with or without discharging sinus (59), pemphigus vulgaris (22), breast abscess (13), postoperative wound infection (10), pyoderma (8), cervical lymphadenitis (5), furuncle (4), and erysipelas (2), [b]Includes pneumonia (30 patients), chronic obstructive pulmonary disease (7), and pleural effusion (4), [c]Includes acute urinary tract infection (13 patients), nephrotic syndrome (2), dysfunctional urinary bladder (2), and ureteric calculi (1), [d]Includes brain abscess (10) and meningitis (2), [e]Includes carcinoma breast (4 patients), hematologic malignancy (4), carcinoma tongue (2), and carcinoma stomach (2), [f]Chronic liver disease (9 patients) and mesenteric abscess (1)

A total of 127 (44.7%) isolates were MRSA. The proportion of MRSA recovered from the OPD patients was 40.5% (32/79), whereas that from the admitted patients was 46.3% (95/205). Frequency of MRSA was highest in the respiratory samples (21/31, 67.7%), followed by urine (16/27, 59.2%), blood (14/25, 56.0%), pus (71/175, 40.5%), and catheter tips (3/8, 37.5%). As compared to the MSSA isolates, MRSA were significantly more resistant to almost all the conventional antimicrobials, except gentamicin, erythromycin, chloramphenicol, and nitrofurantoin [Table 2]. Two hundred and fifty-one (88.3%) isolates were fully susceptible to vancomycin, whereas RVS (including VISA and hVISA) was observed in 33 (11.6%) isolates [Table 2]. Overall, high resistance was observed to penicillin (232/284, 81.7%), erythromycin (177/284, 62.3%), ciprofloxacin (148/284, 52.1%), ofloxacin (146/248, 51.4%), clindamycin (126/284, 44.3%), trimethoprim–sulfamethoxazole (120/284, 42.2%), and levofloxacin (71/284, 25.0%). Resistance was low to gentamicin (15/284, 5.3%), rifampicin (23/284, 8.1%), and doxycycline (27/284, 9.5%), whereas no resistance was observed to nitrofurantoin in the urinary isolates. Multidrug resistance was observed in 158 (55.6%) isolates, and the D-test was positive in 65 (22.8%) isolates [Table 2].

Table 3 shows the MIC distribution of MRSA and MSSA to vancomycin, which reveals that 266 (93.6%) isolates were susceptible to vancomycin, whereas 18 (6.3%, 9 MRSA and 9 MSSA) displayed intermediate resistance. Maximum isolates (41.5%) demonstrated a vancomycin MIC of 2 μg/ml, followed by 1.5 μg/ml (33.8%). Among 18 VISA, 13 (72.2%) had vancomycin MIC 3 μg/ml, 3 (16.6%) had MIC 4 μg/ml, and 2 (11.1%) had vancomycin MIC 6 μg/ml. Among the *S. aureus* strains with vancomycin MIC in the susceptible range (≤2 μg/ml), hVISA phenotype was detected in

15 (5.6%) of the 266 VSSA isolates. Thus, a total of 33 isolates (11.6%, 18 VISA and 13 hVISA) demonstrated RVS, whereas 251 (88.3%) isolates demonstrated full susceptibility to vancomycin. The proportion of isolates with RVS was higher in MRSA (17/127, 13.3%) compared to MSSA (16/157, 10.1%); however, this was not statistically significant. The sources of hVISA were pus, urine, respiratory samples, and blood (11, 2, 1, and 1, respectively). There was a concordance of 97.8% between the results of agar dilution and Etest methods for vancomycin susceptibility. All the *S. aureus* isolates (both MRSA and MSSA) were uniformly susceptible to linezolid (MIC range, 1.5–2 μg/ml), tigecycline (MIC range, 0.064–0.094 μg/ml), and daptomycin (MIC range, 0.19–0.38 μg/ml).

Table 2: Susceptibility profile of *Staphylococcus aureus* isolates (*n*=284) to various antimicrobials and to vancomycin

Antibiotic/or resistance phenotype	Number of resistant isolates, *n* (%)		
	MRSA (*n*=127)	MSSA (*n*=157)	Total (*n*=284)
Penicillin	127 (100)*	105 (66.8)	232 (81.7)
Gentamicin	9 (7.1)	6 (3.8)	15 (5.3)
Doxycycline	18 (14.1)*	9 (5.7)	27 (9.5)
Ciprofloxacin	88 (69.3)*	60 (38.2)	148 (52.1)
Levofloxacin	47 (37.0)*	24 (15.3)	71 (25.0)
Ofloxacin	86 (67.7)*	60 (38.2)	146 (51.4)
Erythromycin	86 (67.7)	91 (57.9)	177 (62.3)
Clindamycin	66 (51.9)*	60 (38.2)	126 (44.3)
Trimethoprim-sulfamethoxazole	74 (58.2)*	46 (29.2)	120 (42.2)
Chloramphenicol	20 (15.7)	29 (18.4)	49 (17.2)
Rifampicin	21 (16.5)*	2 (1.3)	23 (8.1)
Nitrofurantoin#	0	0	0
Linezolid	0	0	0
Daptomycin	0	0	0
Tigecycline	0	0	0
D-test positive	36 (28.3)	29 (18.4)	65 (22.8)
Multidrug resistance	127 (100)*	67 (42.6)	158 (55.6)
Vancomycin			
MIC range (μg/ml)	1-4	0.75-6	0.75-6
MIC_{50} (μg/ml)	2	2	2
MIC_{90} (μg/ml)	2	2	2
VISA	9 (7.1)	9 (5.7)	18 (6.3)
hVISA	8 (6.3)	7 (4.5)	15 (5.3)
RVS	17 (13.3)	16 (10.1)	33 (11.6)

*P<0.05 (significant) for difference between MRSA and MSSA by Chi-square test, #Tested only in urinary isolates. *S. aureus*=*Staphylococcus aureus*, MRSA=Methicillin-resistant *S. aureus*, MSSA=Methicillin-susceptible *S. aureus*, MIC=Minimum inhibitory concentration, VISA=Vancomycin-intermediate *S. aureus*, hVISA=heterogeneous VISA; RVS=Reduced vancomycin susceptibility

Discussion

The study presents a comprehensive data of MRSA and MSSA in a tertiary care hospital in the Eastern part of India, which receives patients not only from the local areas on a primary basis but also large referrals from adjoining districts and states. The study isolates comprised mainly skin and soft tissue (61.6%), followed by respiratory (10.9%) and urine (9.5%) isolates, as has been found in previous observational studies,[25,26] where *S. aureus* was mainly isolated from skin and soft-tissue infections (52.5%–64%), followed by other samples in varying frequency. The study also revealed a prevalence of 44.7% (127 out of 284) MRSA among the *S. aureus* isolates, which is comparable to previously reported studies from India in recent times, with a prevalence of 38.4%–54.8%.[25-27] Further, among 127 MRSA isolates, 91 (71.6%) were MDR, which is similar to the study by Tiwari *et al*.,[27] where among 783 isolates of *S. aureus*, 301 (38.4%) were methicillin resistant, of which 217 (72.1%) were found to be MDR. Similar to previous studies,[12,25,26] the present study also observed that MRSA isolates were significantly more resistant to various antibiotics, such as penicillin, trimethoprim–sulfamethoxazole, fluoroquinolones, erythromycin, and clindamycin as well as they exhibited significantly more multidrug resistance.

As regards vancomycin susceptibility, hVISA was first reported in Japan in 1997 and along with VISA has since been identified worldwide from many countries including the United States, Japan, Australia, France, Scotland, Brazil, South Korea, Hong Kong, South Africa, Thailand, Israel, and others.[3,4] In India, Menezes *et al*.[15] in 2008 reported the emergence of vancomycin-intermediate *Staphylococcus* species in Southern India using the revised guidelines. Of 102 oxacillin-resistant *S. aureus* isolates, one was found to be a VISA strain (MIC 5 μg/ml).[15] In a study from Western region of India, among 58 clinical isolates of MRSA, the prevalence of hVISA was observed to be 6.9% with PAP-AUC and macromethod Etest.[16] In a study from South India (Hyderabad) performed on 358 *S. aureus* isolates only from intensive care units, 285 (79.6%) were identified as MRSA.[28] Sixteen isolates

Table 3: Minimum inhibitory concentration distribution of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* to vancomycin

S. aureus isolates	MIC to vancomycin (μg/ml) by Etest							
	0.75	1	1.5	2	3	4	6	Total
MRSA	0	23	44	51	7	2	0	127
MSSA	2	27	52	67	6	1	2	157
Total (%)	2 (0.7)	50 (17.6)	96 (33.8)	118 (41.5)	13 (4.5)	3 (1.1)	2 (0.7)	284

S. aureus=*Staphylococcus aureus*, MRSA=Methicillin-resistant *S. aureus*, MSSA=Methicillin-susceptible *S. aureus*, MIC=Minimum inhibitory concentration

showed an MIC range between 4 and 8 μg/ml, indicating vancomycin-intermediate resistance, and seven isolates showed an MIC range between 16 and 64 μg/ml, indicating VRSA.[28] PCR amplification for *vanA* among the seven VRSA showed that six contained *vanA* and one was negative for *vanA*.[28] Apart from these, two studies from North India have observed the presence of VISA and hVISA in their setup.[18,19] In Karachi (a neighboring region of India), of 450 *S. aureus* isolates, 174 (38.6%) were found to be MRSA.[29] One isolate was found to be vancomycin resistant (MIC 32 μg/ml), whereas four isolates had intermediate resistance, with two strains having MIC of 16 μg/ml and two having MIC of 8 μg/ml.[29] Thus, the hVISA and VISA rate found in the current study is similar to the previously reported studies in India and demonstrates the emergence of RVS in yet another region (Eastern) of India. Further, though the hVISA rate found in the current study appears low (6.3% of MRSA and 4.5% of MSSA), the total rate of RVS (hVISA and VISA) is moderately high (11.6% overall, 13.3% in MRSA, and 10.1% in MSSA). Apart from MRSA, several studies have found RVS in MSSA isolates, which has been observed to be associated with poorer outcomes and increased complications.[5,7,8] One study has shown that the development of intermediate vancomycin susceptibility in MSSA may result in increased tolerance to several other classes of antistaphylococcal antibiotics.[30] The clinical implications of RVS observed in MSSA isolates in our setup needs to be investigated further by prospective cohort studies.

Conclusion

The present study shows that a moderately high percentage of *S. aureus* demonstrated RVS, which may limit its usefulness in MRSA and may be associated with increased complications in MSSA infections. Linezolid, tigecycline, daptomycin, and doxycycline may be considered as alternatives for the treatment of isolates with reduced susceptibility to vancomycin. In view of increasing glycopeptide resistance, the susceptibility status of vancomycin along with other antibiotics among clinical S. aureus isolates should be investigated periodically.

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Conflicts of interest
There are no conflicts of interest.

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